Reasons and Technology for Controlling Excessive Methanogenesis during Remedial Action

Remediation Session: Thursday May 17, 2018

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Provectus Environmental Products, Inc - Freeport, IL

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Presentation Outline

- What is the Problem with Methane?
- Antimethanogenic Reagents (AMRs)
- AMR-Based Environmental Biotechnologies
  - **Provect-CH4®** Methane Inhibitor
  - **ERD-CH4® Ole Ego** Liquid, Antimethanogenic ERD Reagent
  - **Provect-IR®** Solid, Antimethanogenic ISCR Reagent
  - **Provect-IRM®** Antimethanogenic ISCR/Heavy Metal Immobilization Reagent
  - **AquaGate-CH4®**Antimethanogenic Reactive Cap
  - **EZVI-CH4™** Antimethanogenic Reagent for DNAPL
- Case Study: Dry Cleaner in Urban Setting
- Summary and Conclusions

Why Add Carbon/ZVI to Reduce ORP?

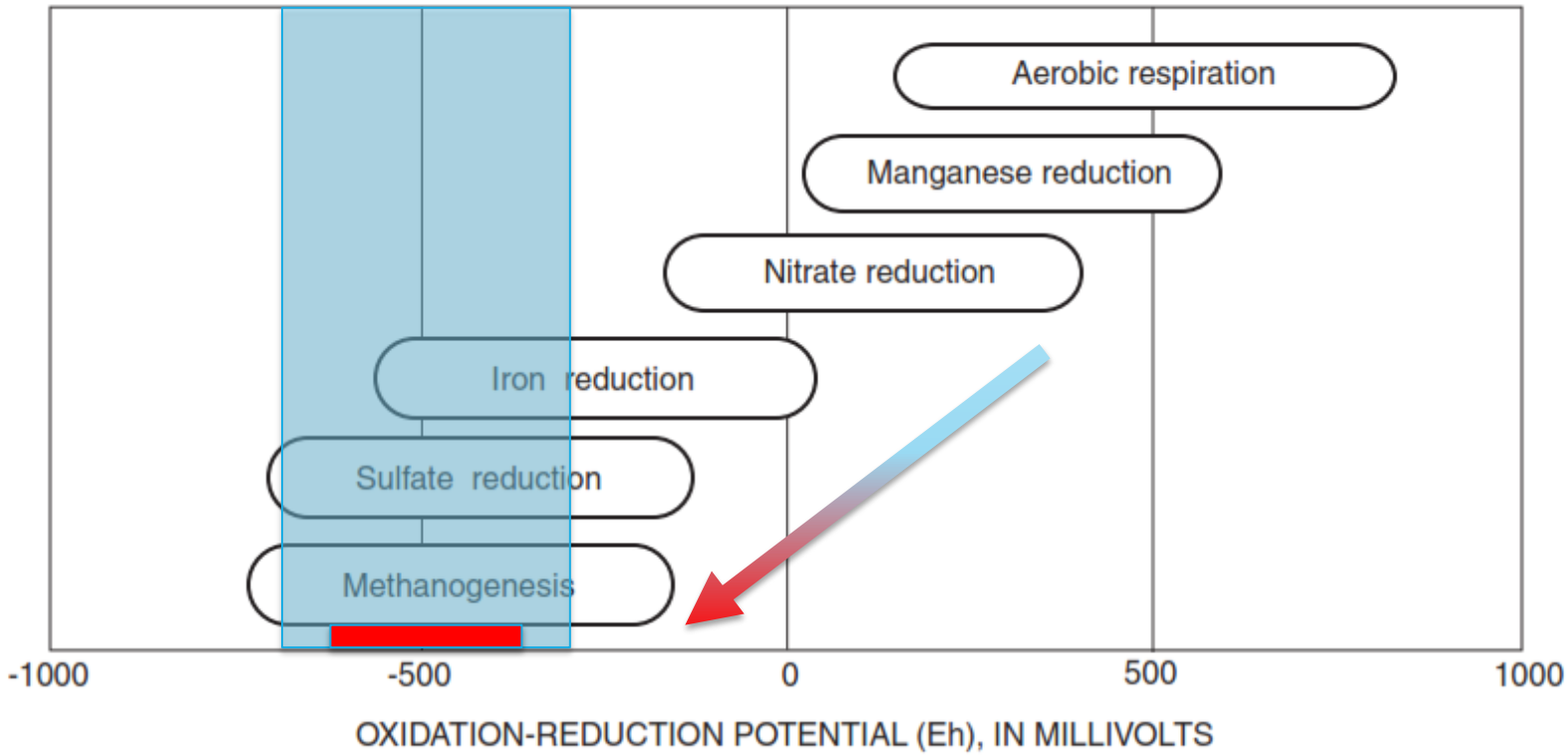


Figure 2. Oxidation-reduction potentials for selected microbial processes. (Modified from Stumm and Morgan, 1981.)

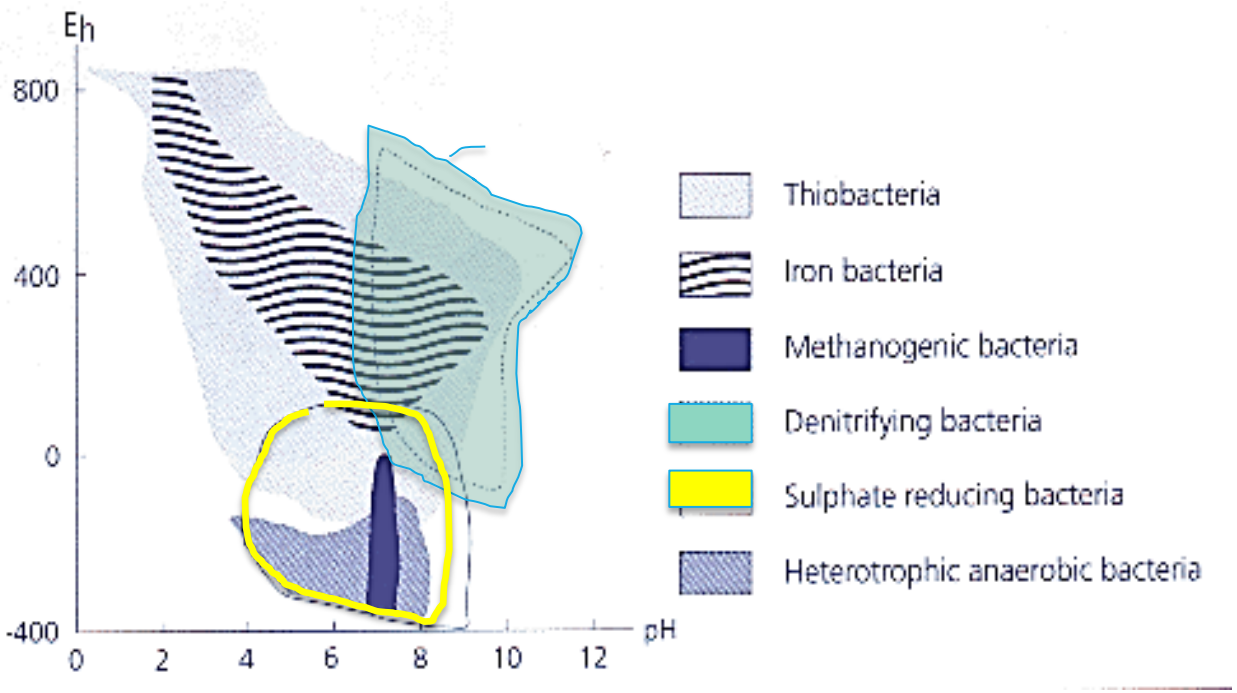
What is a Methanogen?

- Methanogens are microorganisms that produce methane
- Methanogens are Archaea (Woese and Fox, 1977) and hence, from a genetic perspective, *Dehalococcoides mccartii* are as different from methanogens as you are.
- Methanogens are often dominant as compared to DHC spp. and acetogens: averaging 2% to 15% of all soil microbes (Bates, *et. al.,* 2011)

  When biostimulated populations of DHC can rise to >10^8 cells/L, but Archaea populations can be still be orders of magnitude greater in number
- Methanogens are important members of synergistic, fickle anaerobic communities = we need some

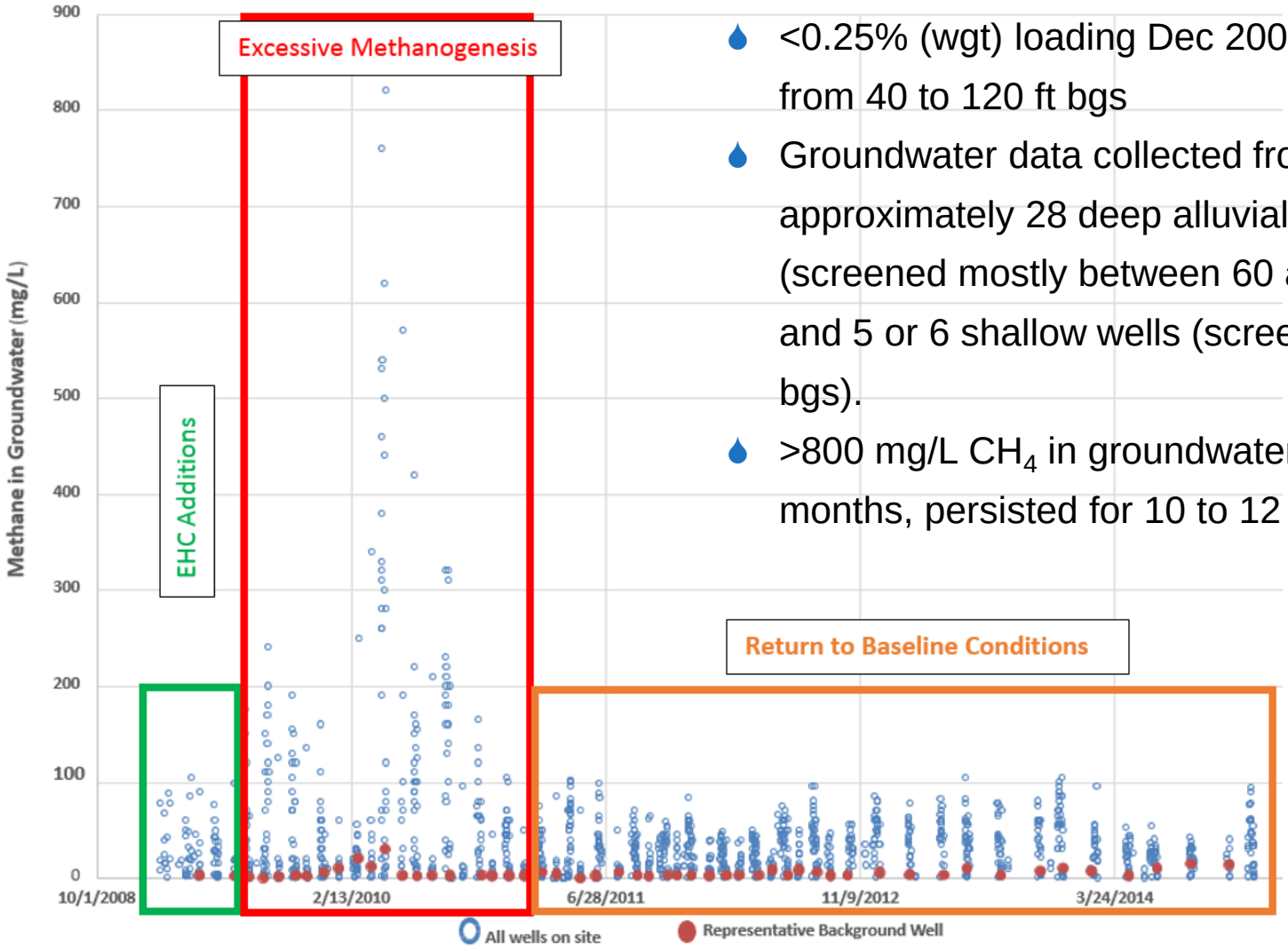
Idealized Eh pH Ranges for Microbial Growth

Microbe	Doubling Times
Dehalococcoides spp.	24 to 48 hours
Methanogens with cytochromes	10 hours
Methanogens without cytochromes	1 hour



Zajic, 1969. Sigma Aldrich

Example of Excessive Methanogenesis



- <0.25% (wgt) loading Dec 2008-May, 2009 from 40 to 120 ft bgs
- Groundwater data collected from approximately 28 deep alluvial wells (screened mostly between 60 and 160 ft bgs) and 5 or 6 shallow wells (screened 25-35 ft bgs).
- >800 mg/L CH_4 in groundwater after 6 months, persisted for 10 to 12 months

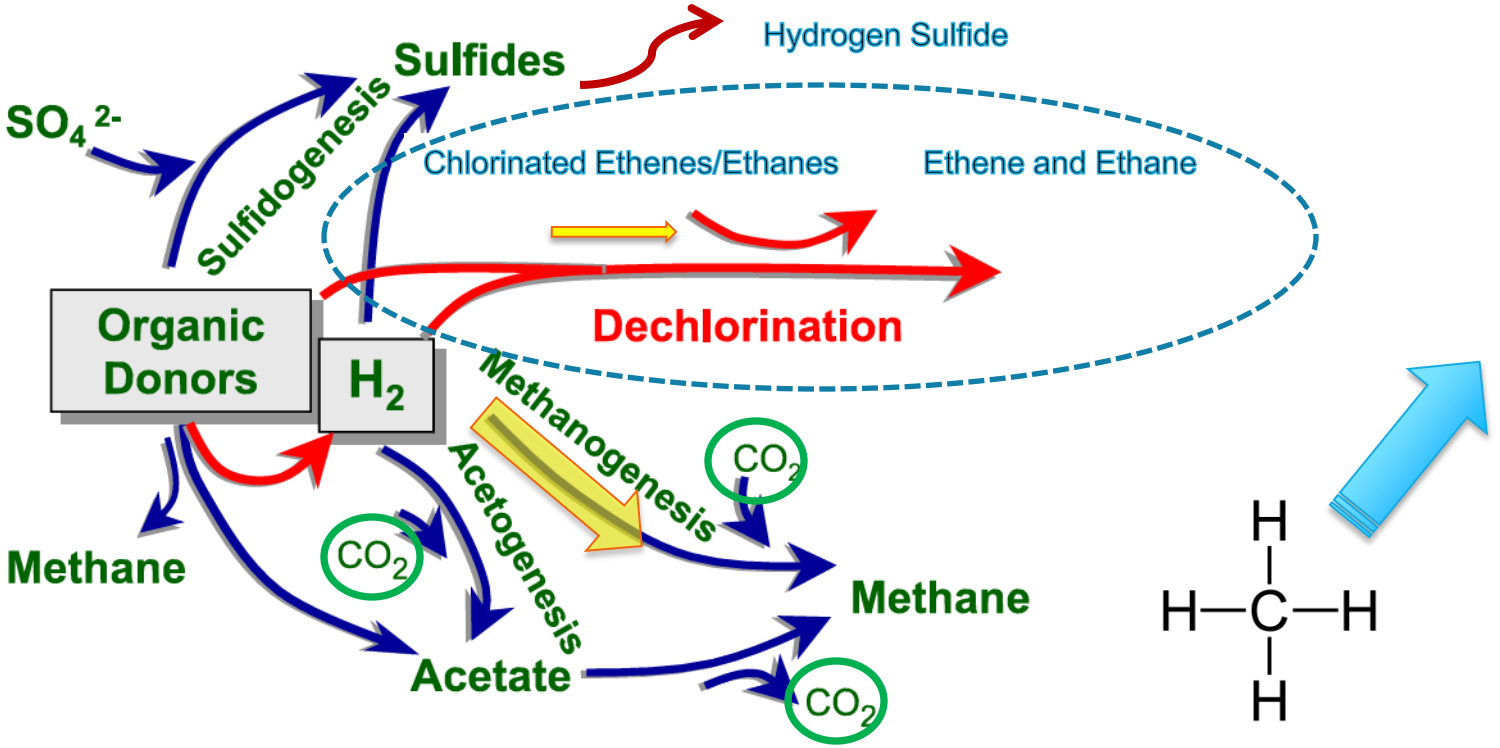
Source: Peale et al, 2009; 2010

Excessive CH_4 16 months post EVO

Source: US DOD, 2016; 2017

Hydrogen is the Currency

- **Where Does it Go? = Cost and Efficiency Issues**: Methanogens dominate anaerobic ecosystems and they can hinder dechlorination by competing for H_2 with dechlorinating bacteria (Yang and McCarty, 1998; yellow arrows modified by Provectus).



What is The Problem With Methane?

- **#1 Cost and Efficiency Issues**: Production of methane is a direct indication that hydrogen generated from the electron donor amendments was used by methanogens instead of the target microbes (*e.g., Dehalococcoides spp*.), substantially reducing application efficiency.

Constituent	Groundwater Concentration (mg/L)	Molecular Weight (g/mol)	Moles of H_2 to Reduce Mole Analyte	Moles of H_2 Acceptor In Treatment Area
Contaminant Electron Acceptors (To End Product Ethene)				
Tetrachloroethene (PCE)	10.0	165.8	4	1,393
Trichloroethene (TCE)	7.0	131.4	3	364
cis-1,2-Dichloroethene (cDCE)	0.0	96.9	2	0
Vinyl Chloride (VC)	0.0	62.5	1	0
			Complete Dechlorination (Soil+Groundwater) Subtotal	**1,757**
Native Electron Acceptors				
Dissolved Oxygen	9.0	32	2	199
Nitrate (as Nitrogen)	9.0	62	3	682
Sulfate	50.0	96.1	4	736
Fe^{+2} Formation from Fe^{+3}	20.0	55.8	0.5	63
Mn^{+2} Formation from Mn^{+4}	10.0	54.9	1	64
			Baseline Geochemistry Subtotal	**1,745**
Hydrogen Waste for Methane Formation				
Methane Formed	20.0	16	4	**1,769**
			Initial Treatment Area Hydrogen Usage	**5,271**

Even in a highly oxidized setting with relatively high total concentrations of PCE and TCE, generating just 20 mg/L of methane constitutes **greater than 33%** of the total amendment consumption based on moles of H_2.

What is The Problem With Methane?

- **#2 *Potential* Health and Safety Issues (*in Some Situations*):**

✓ Methane is explosive, with an LEL of 5% and an UEL of 15%.

- Subsurface fires next to an industrial facility at a site in the Midwest USA immediately following the application of a conventional ISCR reagent;
- Generation of up to 23% methane in soil gas immediately adjacent to a public church in North Carolina (with sustained methane production >LEL for 8 to 9 months) from an excavated area treated with conventional ISCR reagent;
- Requirement to install an air sparge/SVE contingency system at a site in northern Indiana due to methane accumulation associated with an application of emulsified oil;
- Changes in aquifer flow dynamics and inactivation of an *in situ* injection system due to methane production and extensive biomass generation following repeat applications of molasses for ERD at a site in Rio De Janeiro, Brazil; and
- Delayed occupancy of a newly developed, high-rise residential complex in Sao Paolo, Brazil due to presence of elevated methane in soil gas following the use of conventional ISCR reagent.

What is The Problem With Methane?

- **New and Emerging Regulatory Issues**:

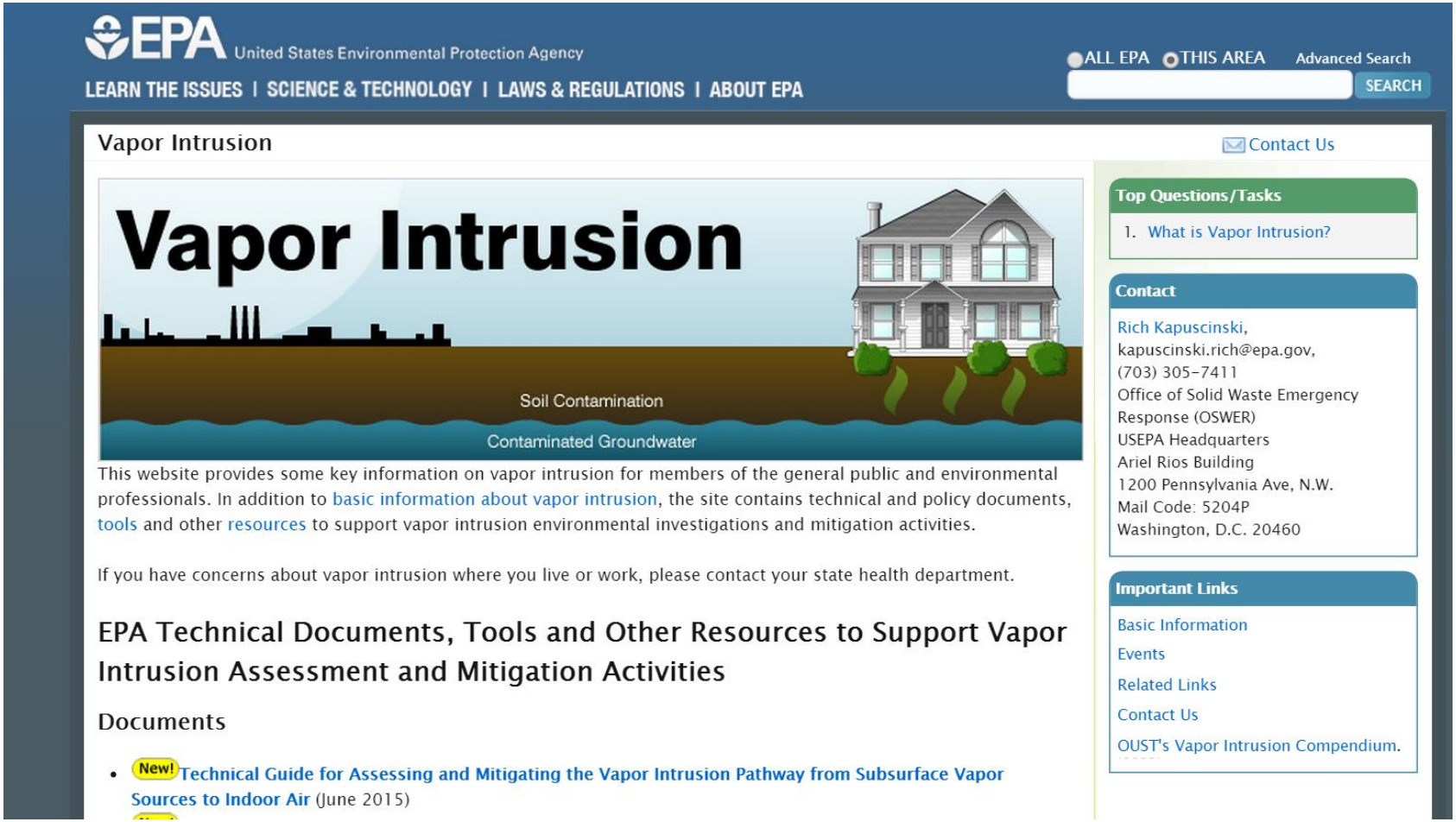
✓ State specific regulations for methane in groundwater have been promulgated, with others pending for soil gas and indoor air: CA, FL, IL, IN, MN, NJ, NC

✓ For example, current regulations for methane in groundwater vary from *ca*. 10 ppm to 28 ppm CH_4 (IDEM, 2014).

✓ Notably, several ERD projects which intended to use liquid carbon (emulsified oils) sources have failed to receive regulatory approval due to issues associated with excessive production of methane during previous technology applications As a result, many remedial practitioners proactively design contingencies for conventional ERD/ISCR implementation in the event that methane exceeds a threshold level ranging from 1 ppm to 10 ppm in groundwater.

✓ These contingencies often entail expensive and extensive systems for capturing methane in soil gas/vapor via SVE systems and subsequently treating the vapors.

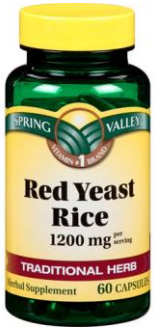
What Does the US EPA Say?

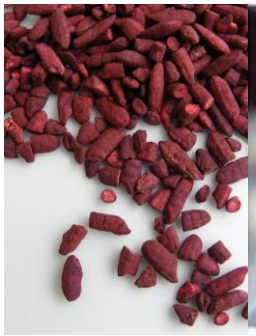


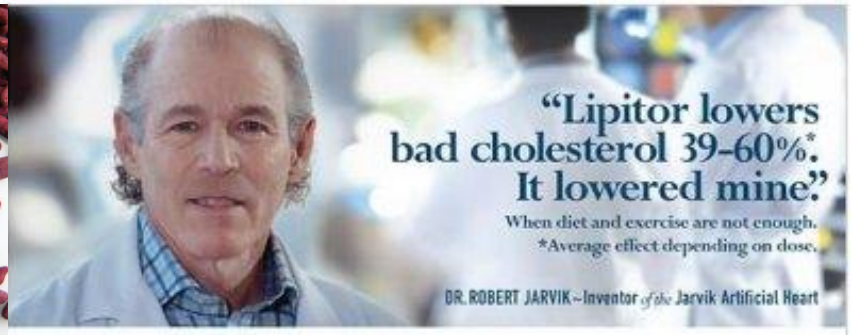
- http://www.epa.gov/oswer/vaporintrusion/

What is Red Yeast Rice (RYR) Extract?

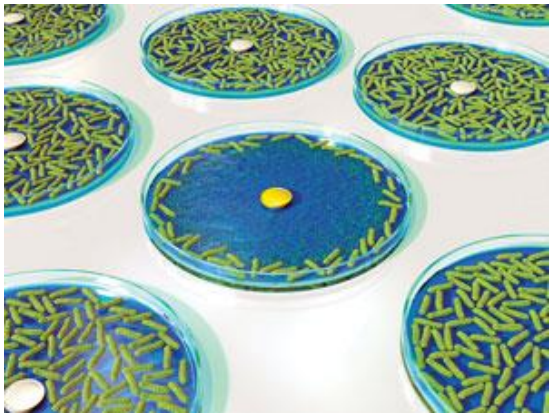
- RYR extract is a substance extracted from rice that has been fermented with a yeast called *Monascus purpureus*.
- RYR extract contains a number of natural statins - most importantly, Monacolin K - otherwise known as Lovastatin® / Lipitor® /etc.
- In addition to Monacolin K, RYR also contains 9 other statins, mono-unsaturated fatty acids, vitamins and other nutrients that will effectively stimulate anaerobic bacteria.
- RYR is used as a food coloring, food additive and preservative, and is widely consumed directly by humans.

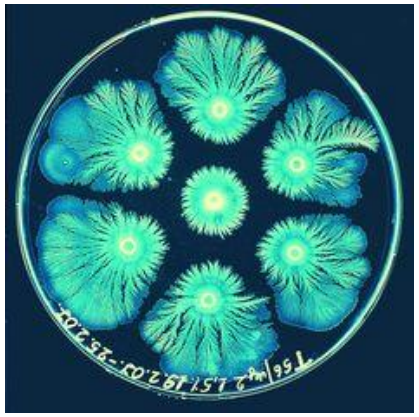




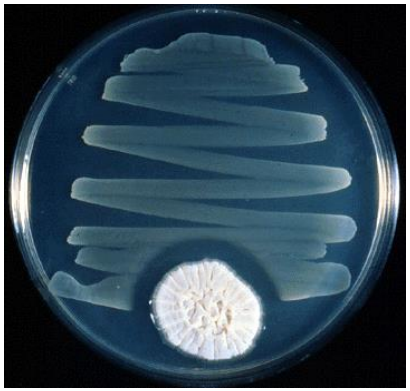


Why Does RYR Produce Statins?



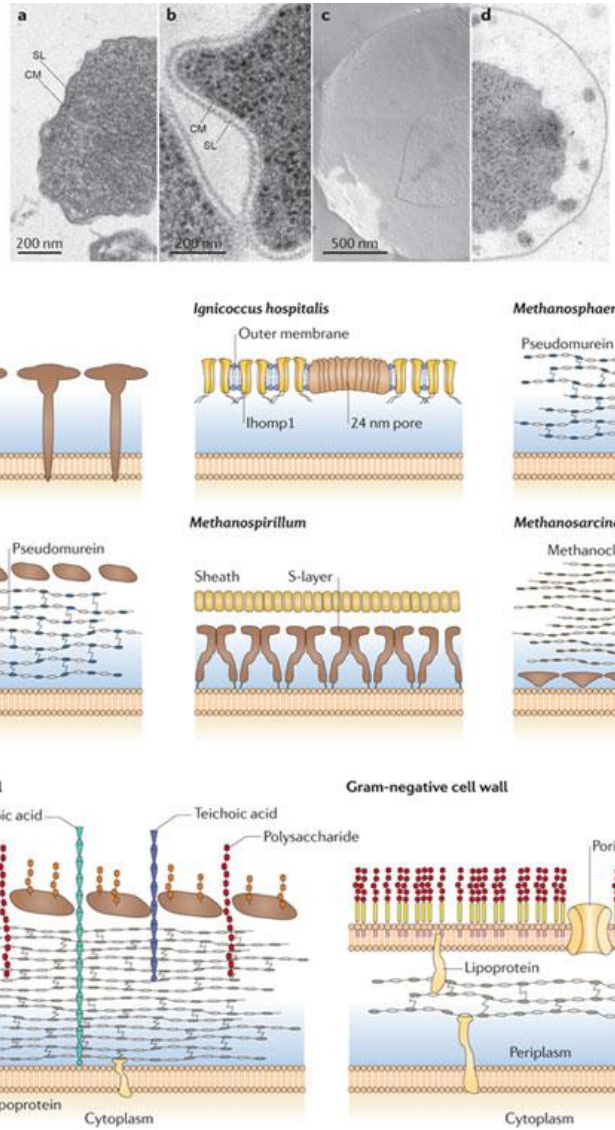


- Many microorganisms produce bioactive compounds that inhibit / regulate the growth and development of other organisms
- Example, antibiotics such as penicillin which is produced by mold of *Penicillium* genus

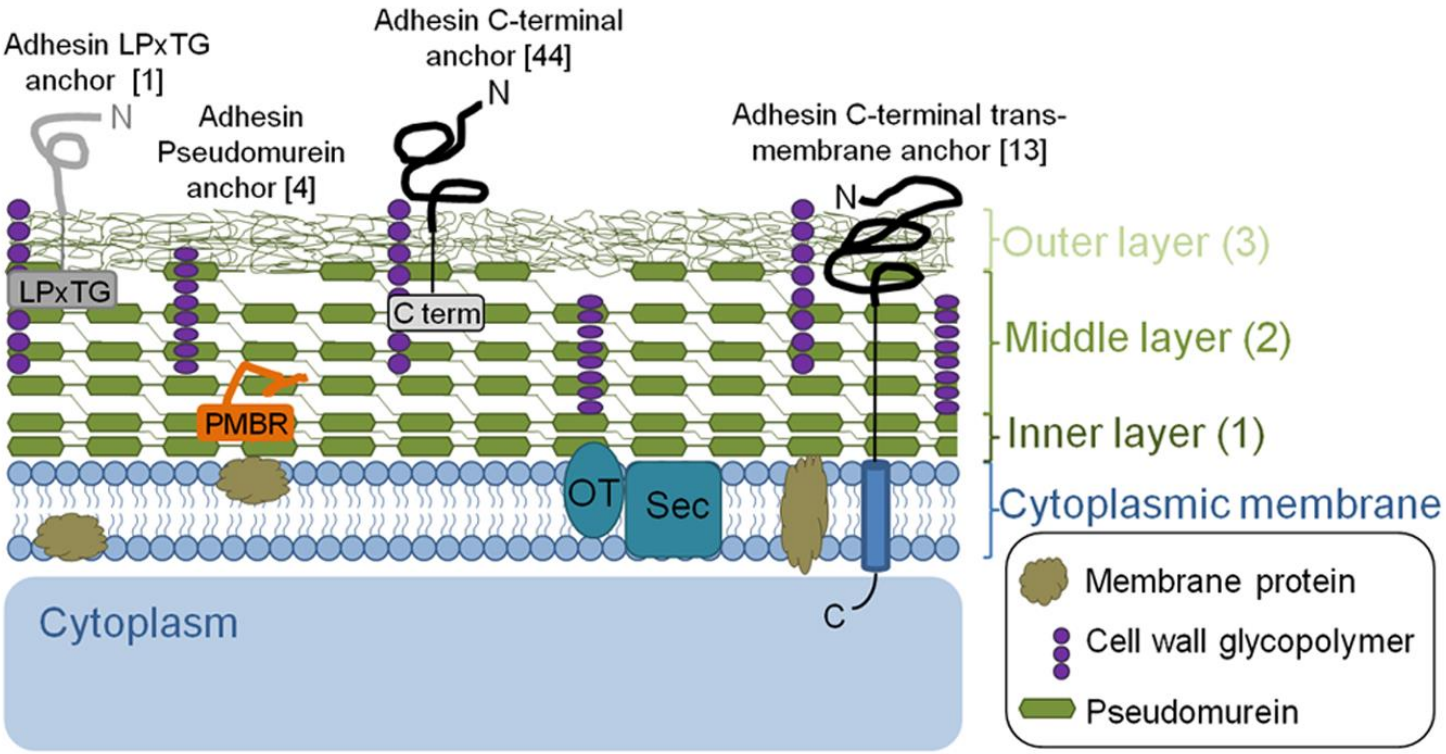

How Does RYR Control Methanogens?

- Bacteria cell walls contain peptidoglycan (murein).
- Methanogens cell walls contain pseudomurein.
- Pseudomuerin is biosynthesized via activity similar to that of 3-hydroxyl-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, which is a key enzyme in the cholesterol biosynthesis pathway in humans (Alberts *et al.*, 1980).



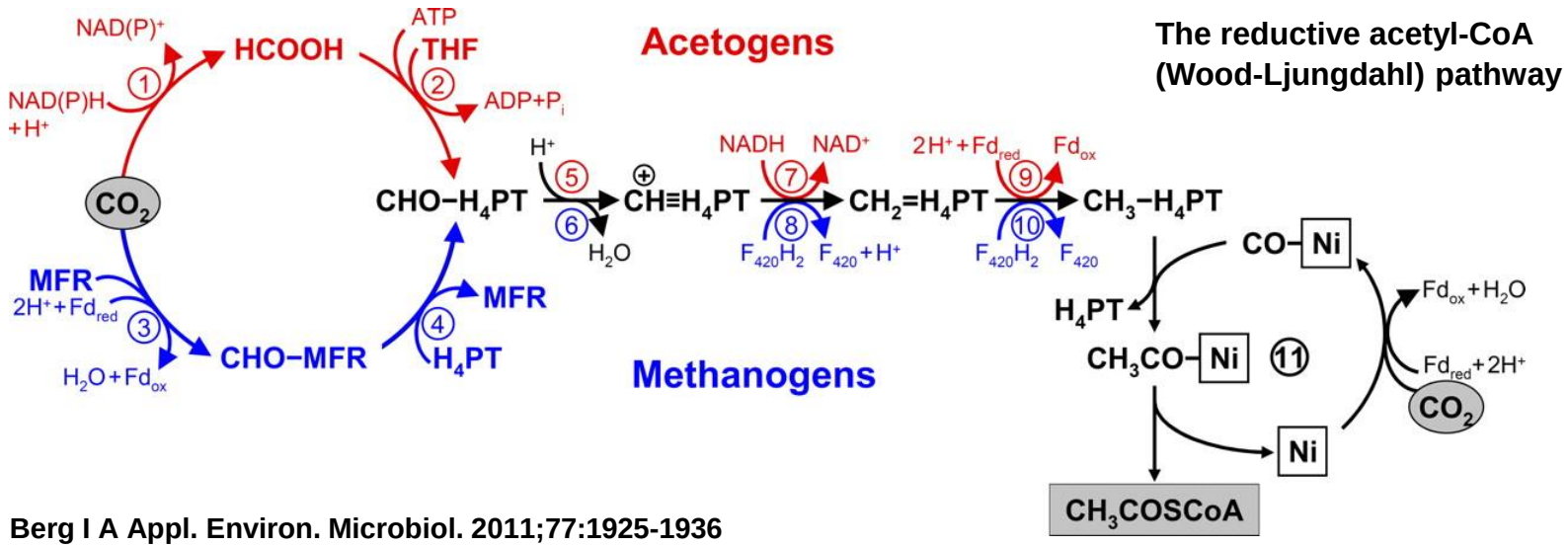
How Does Provect-CH4 Control Methane?

- In the presence of a Monacolin K and other statins in Provect-CH4™ HMG-CoA reductase is inhibited, pseudomurein ( ) biosynthesis pathway is interrupted, and methanogens are restricted from growth, development and proliferation.



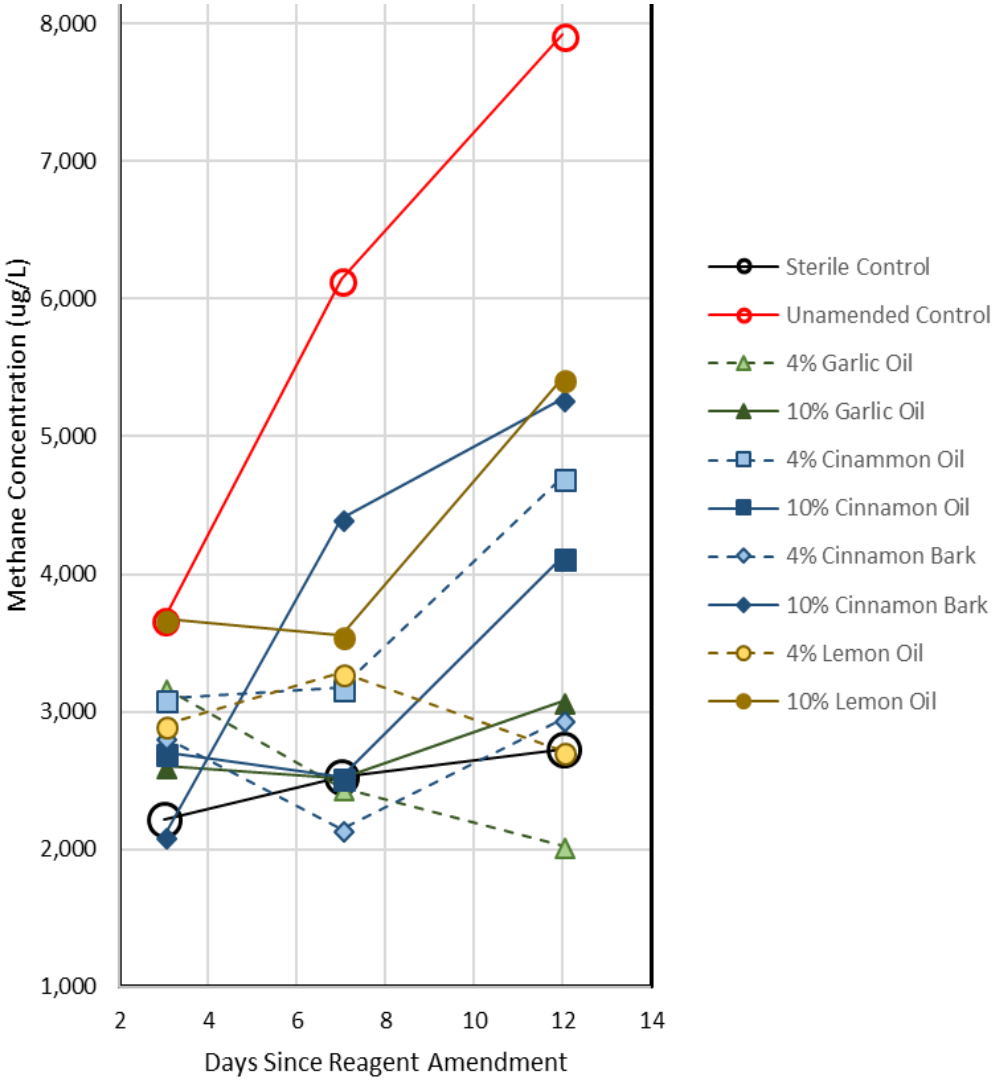
How Does Provect-CH4 Control Methane?

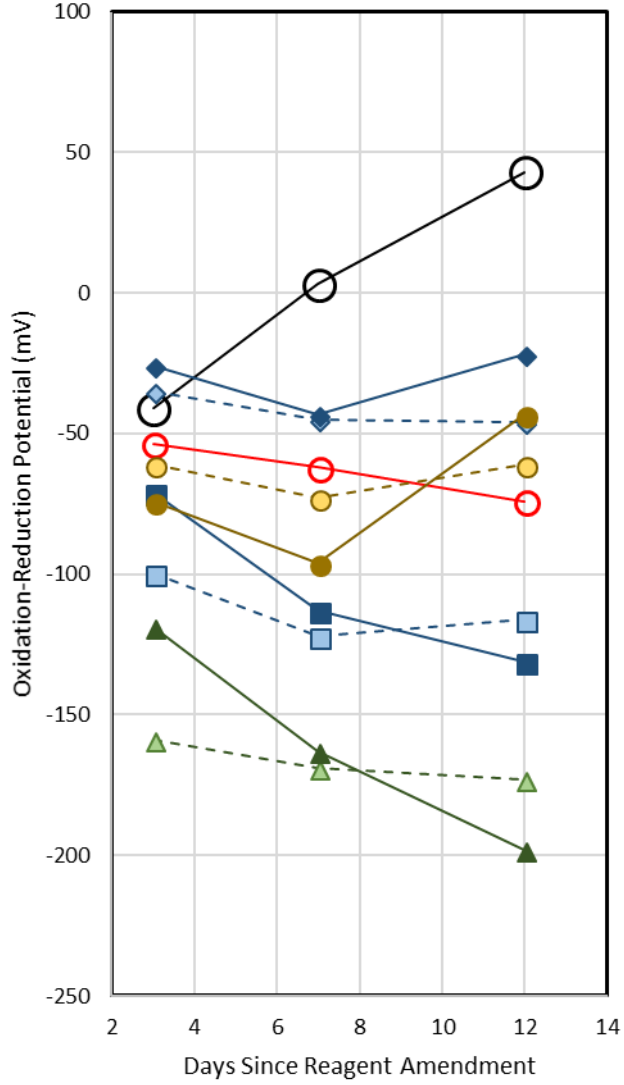
- Other compounds also interrupt F420 coenzyme synthesis and other systems unique to methanogens which restrict their growth and proliferation.
- Because Archaea are so different from other microbes, these inhibitory effects are selective to methanogens and are not observed in microbes that are typically associated with: i) catabolism of organic contaminants (such as *Pseudomonas spp*.) and/or, ii) halo-respiration/biodegradation of chlorinated solvents (such as *Dehalococcoides spp*).



Berg I A Appl. Environ. Microbiol. 2011;77:1925-1936

Oil-Based AMRs: Initial Studies



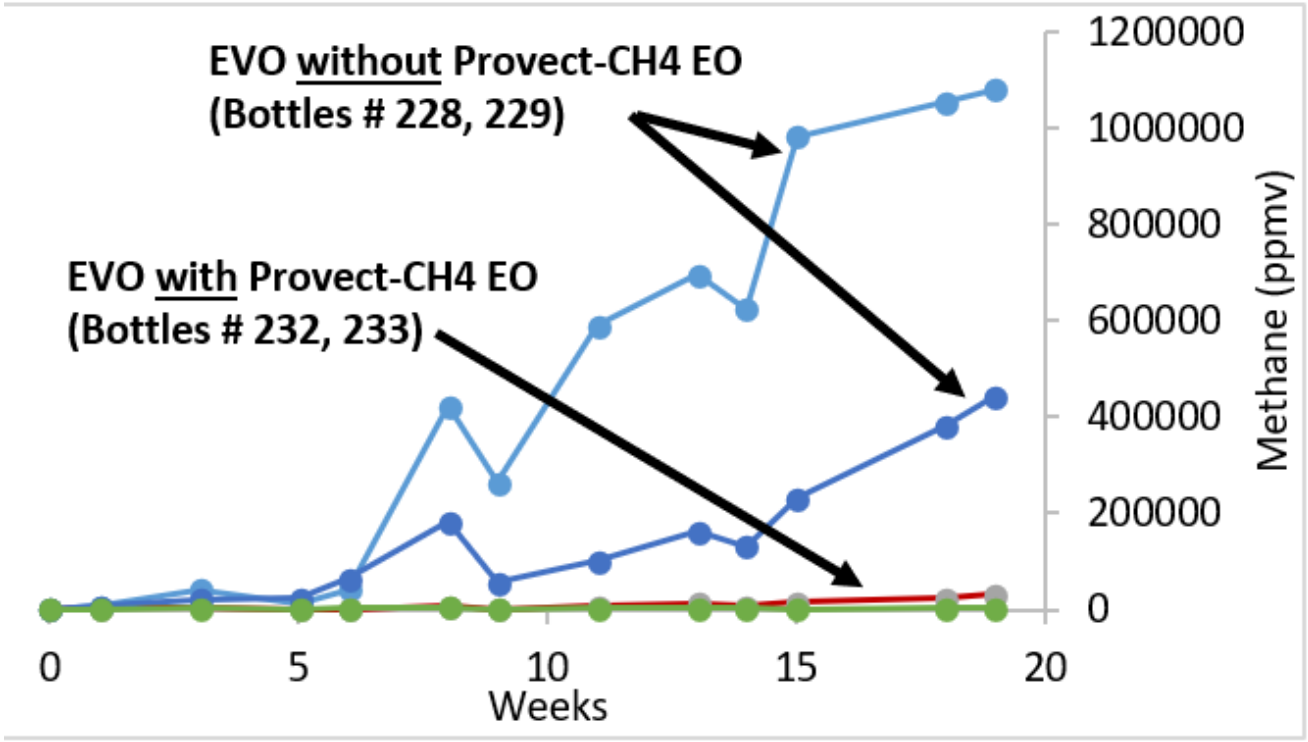


EGO AMR Studies Clemson Univ.

- 50 g aquifer solids
- 30 ml groundwater
- 40 mM EVO

(ca. 8,000 ppm TOC)

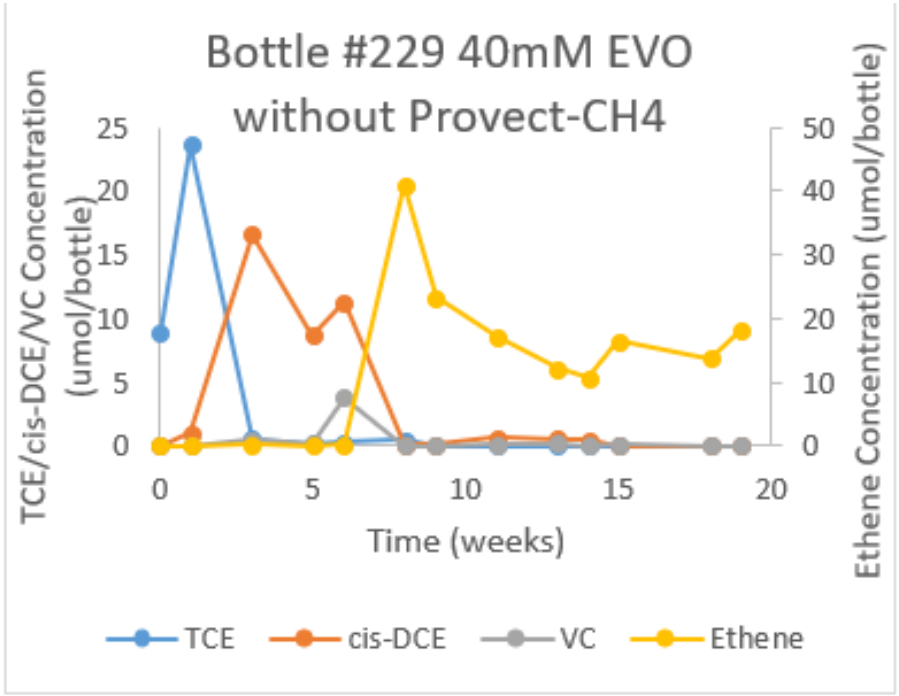
- +/- 250 ppm AMR
- 10 µmol TCE
- DHC added

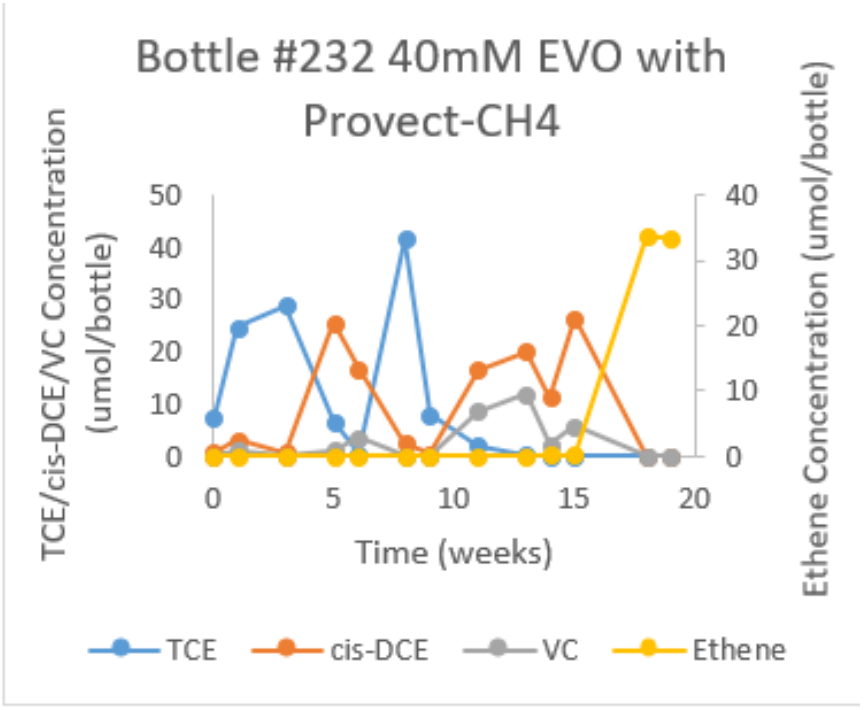


19 weeks incubation cumulative methane production

- **No AMR** CH4 >100,000 ppmV closed headspace; > 900 µmol (> 480 ppm) in water
- **+ AMR** CH4 <10,000 ppmV closed headspace; < 10 µmol (< 5 ppm) in water
- **90 to >99% reduction in methane production with Provect-CH4 Ego**

EGO AMR Studies Clemson Univ.

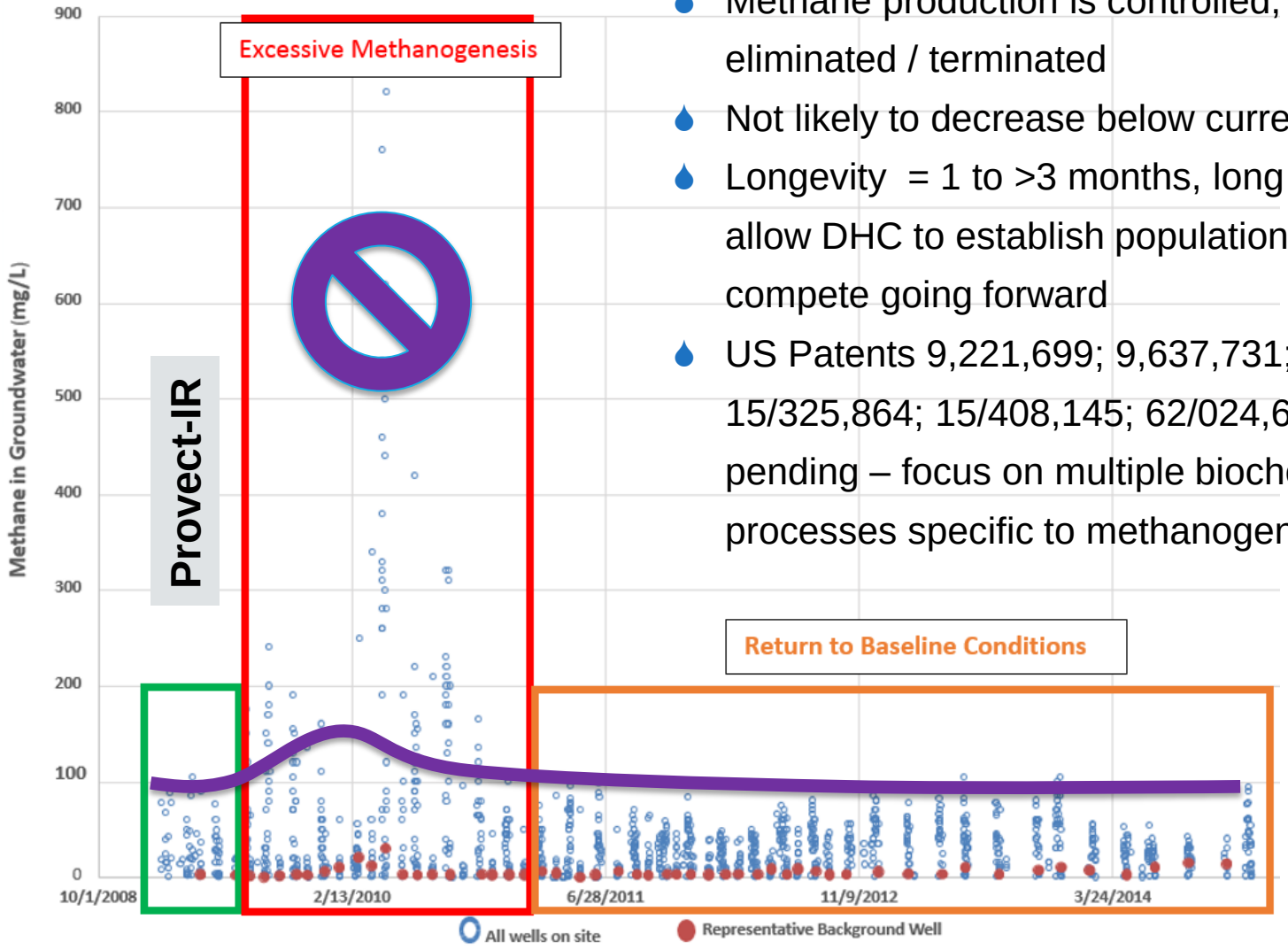




19 weeks incubation

- **No significant difference in rate of TCE dechlorination with or without AMR**

Controlled Methanogenesis AMR



- Methane production is controlled, not eliminated / terminated
- Not likely to decrease below current conditions
- Longevity = 1 to >3 months, long enough to allow DHC to establish populations and better compete going forward
- US Patents 9,221,699; 9,637,731; 15/269,903; 15/325,864; 15/408,145; 62/024,640; patents pending – focus on multiple biochemical processes specific to methanogens

Provect-ERD CH4® Ole Ego™

- Liquid, AMR ERD substrate
- 60 to >90% FC
- Contains RYR and EGO

Photograph 1. Provect-ERD CH4™ 15:1 Water:Oil (Left), 85% Carbon + 4% AMR Self-Emulsifiable Oil Concentrate (Middle), and 85% Carbon Self-Emulsifiable Oil Concentrate, no AMR (Right).

Product Data

The materials are all combined at our own manufacturing facilities in the USA (and Europe) at proportions and formulations optimized for a given site. ERD-CH4™ is manufactured using 100% food grade ingredients that provide fast- and slow-release characteristics. Provect-CH4® antimethanogenic reagent (AMR) is typically added at three to five weight percent of the mass of the fermentable carbon. The common dosage of ERD-CH4 provides groundwater concentrations of 1,000 to 3,000 ppm TOC plus a minimum 150 ppm of AMR within the targeted treatment area.

Color	Translucent Yellow
Density (lbs. / gal)	7.75 to 8.46 (varies based on AMR)
Physical State	Liquid
Odor	Earthy
Viscosity (Brookfield, 30 rpm @25°C)	50-100 cps
pH – 1% w/v in water	7.3

Oil Sample	Al	P	S	Zn	Fe	Mg	Ca	Na	K
Self-Emulsifiable Vegetable Oil	1.2 to 1.3	1,265 to 1,751	17.2 to 28.2	19.6 to 39.7	1,4 to 2.3	138 to 143	135 to 187	15.4 to 15.9	507 to 954

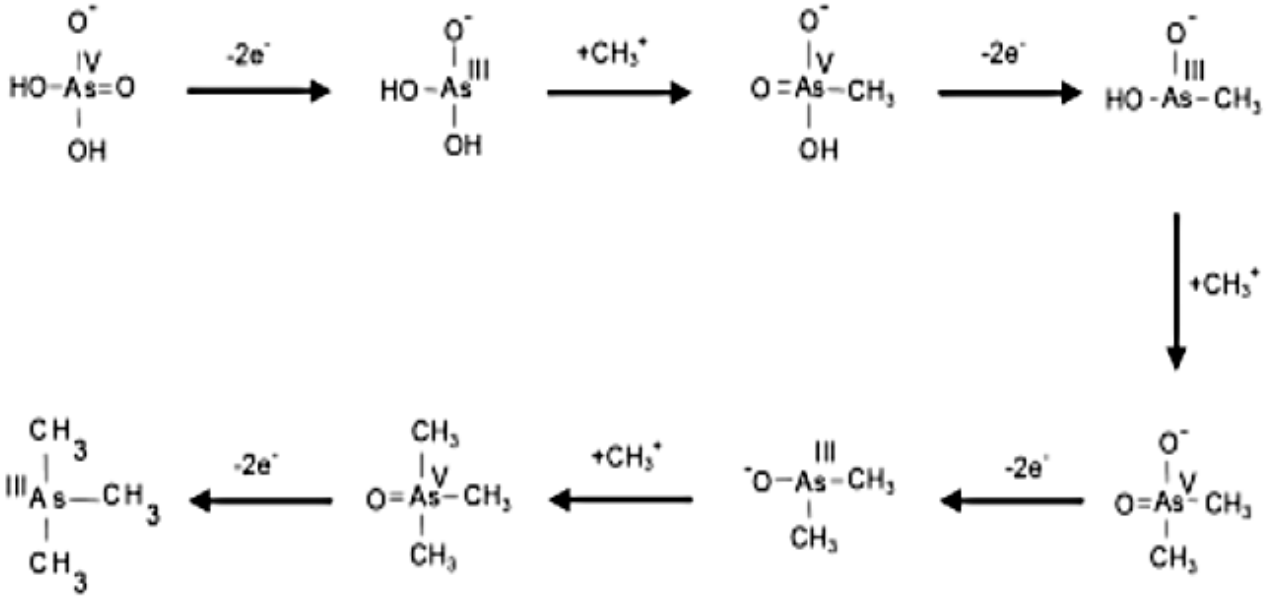
- **All units mg/kg oil**

Provect-IR Solid, AMR ISCR Reagent

- Provect-CH4® AMR Technology
- Multiple, Complex, Hydrophilic, Timed-Release organic carbon source (plant materials, kelp, Ca propionate) @ 390 g H donor / lb product
- 15% to 85% (wgt) ZVI particles (from 3 to >495 micron)
- Integrated vitamins, minerals and nutrients (yeast extract) specially selected for anaerobes
- Chemical oxygen scavenger to maintain ZVI
- Package in 50 lb safety bags or 2,000 lb supersacs.

Methanogens (and others) Methylate Metals

- Provect-IRM limits the number and activity of methanogens hence the targeted metal contaminants are more able to participate in the desired stabilization reactions.
- Moreover, the overall toxicity of the site is not increased via the generation of methylmetal(loids) as a consequence of the treatment process (example – biomethylation of arsenate).

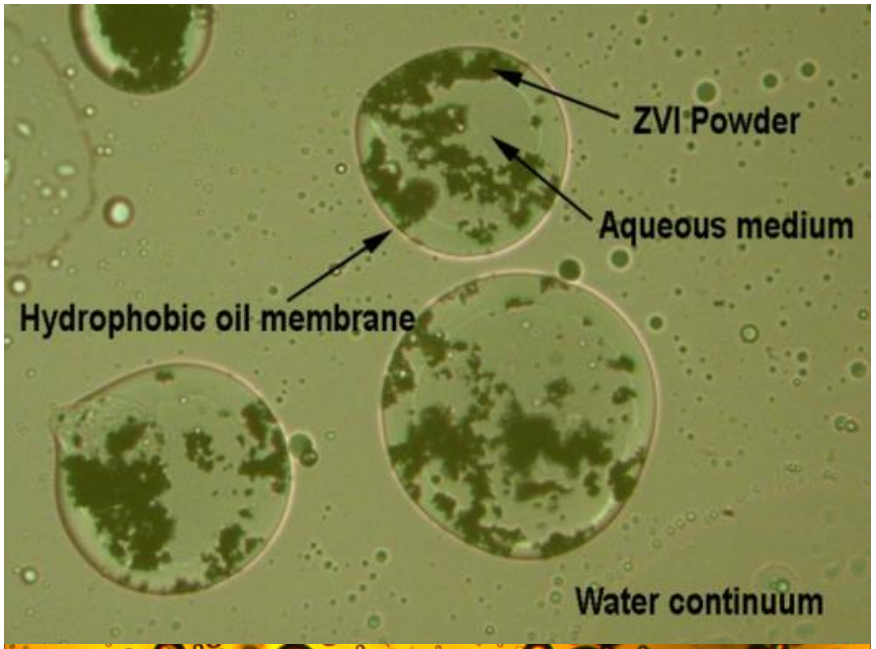


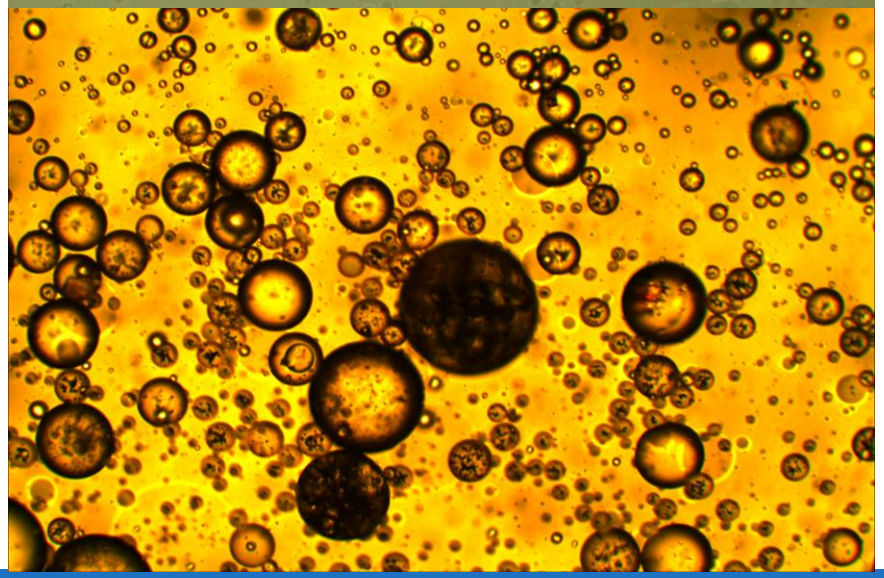
Challenger mechanisms for biosynthesis or Arsenate (Challenger, 1945)

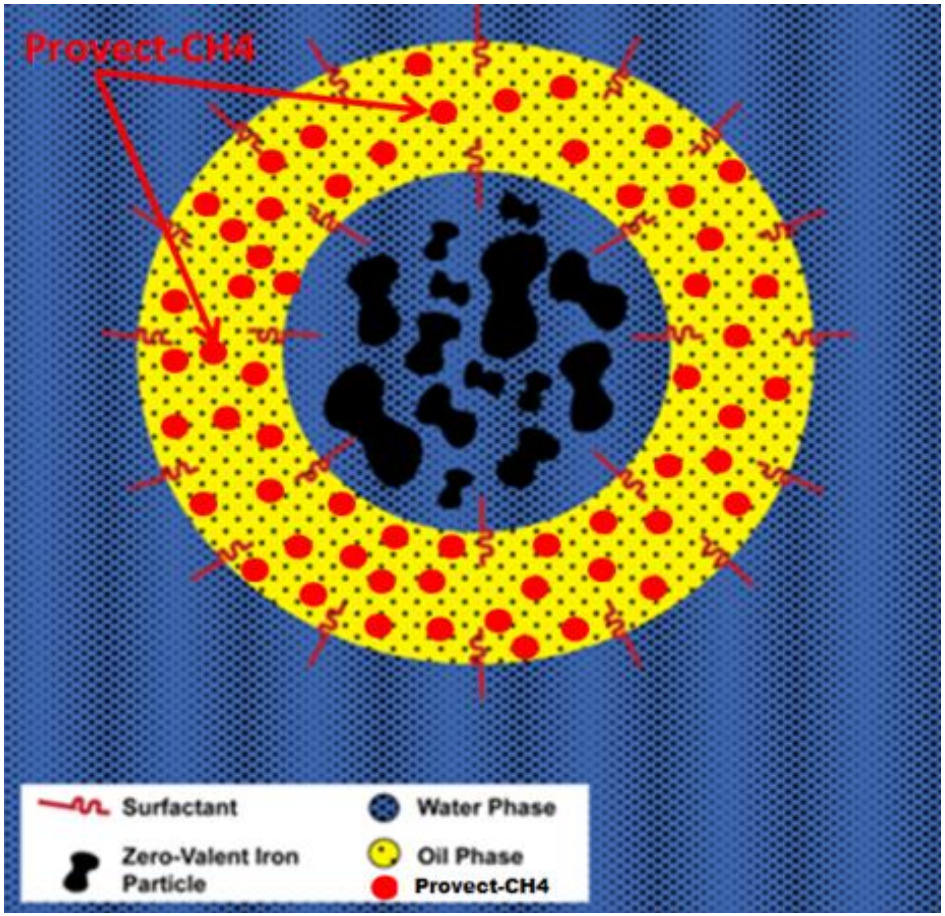
Provect-IRM Solid, Antimethanogenic ISCR Reagent for Heavy Metals

- **Formulated based on site requirements**
- Provect-CH4 AMR Technology
- Multiple, Complex, Hydrophilic, Timed-Release organic carbon source (plant materials, kelp, Ca propionate)
- Inorganic S source, if needed
- 15% to 85% (wgt) ZVI particles (from 3 to >495 micron)
- Integrated vitamins, minerals and nutrients (yeast extract) Chemical oxygen scavenger to maintain ZVI
- Package in 50 lb safety bags or 2,000 lb supersacs.

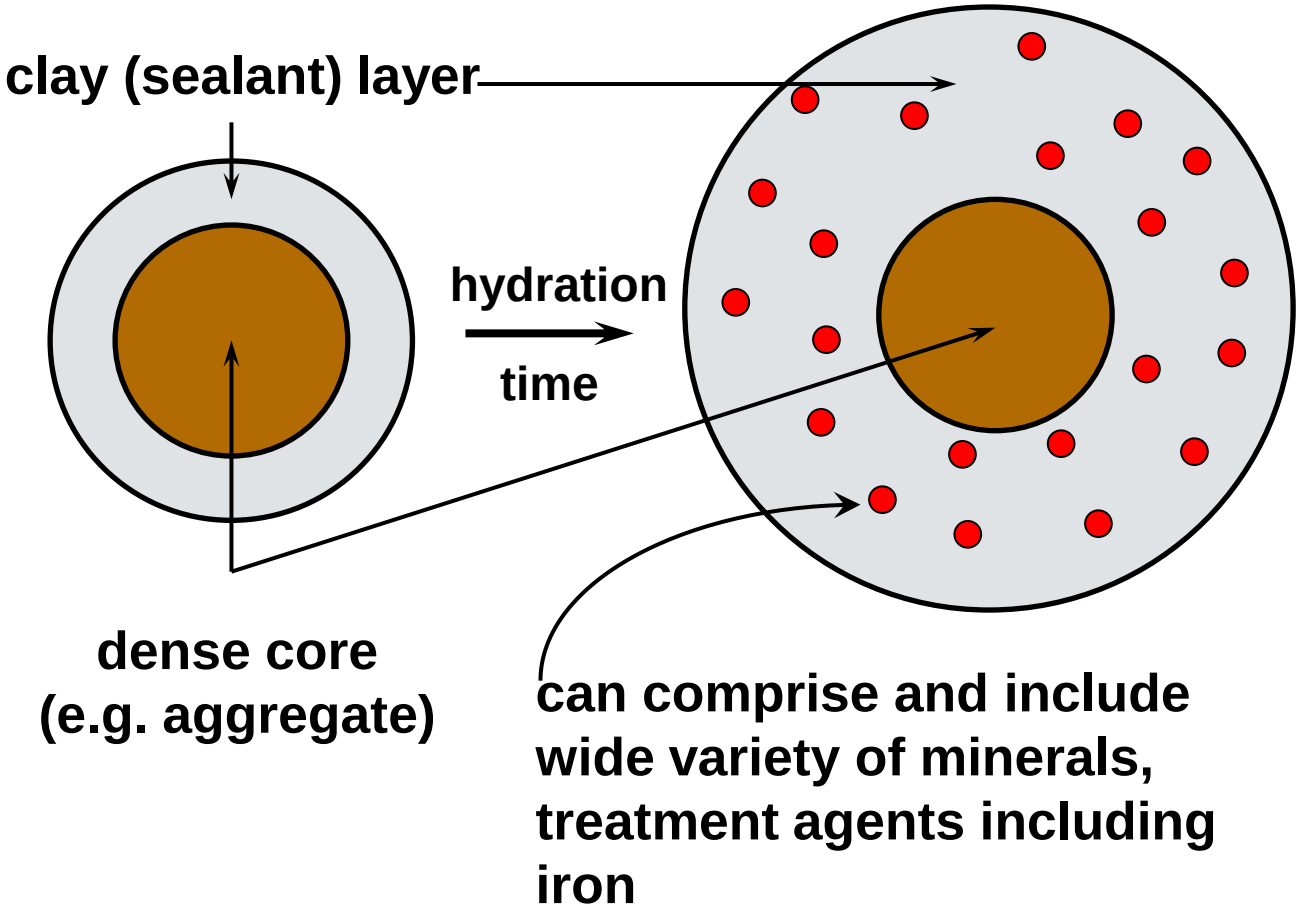
EZVI-CH4™ AMR DNAPL Technology







AquaGate®-CH4 AMR Capping



Case Study: Provect-IR®

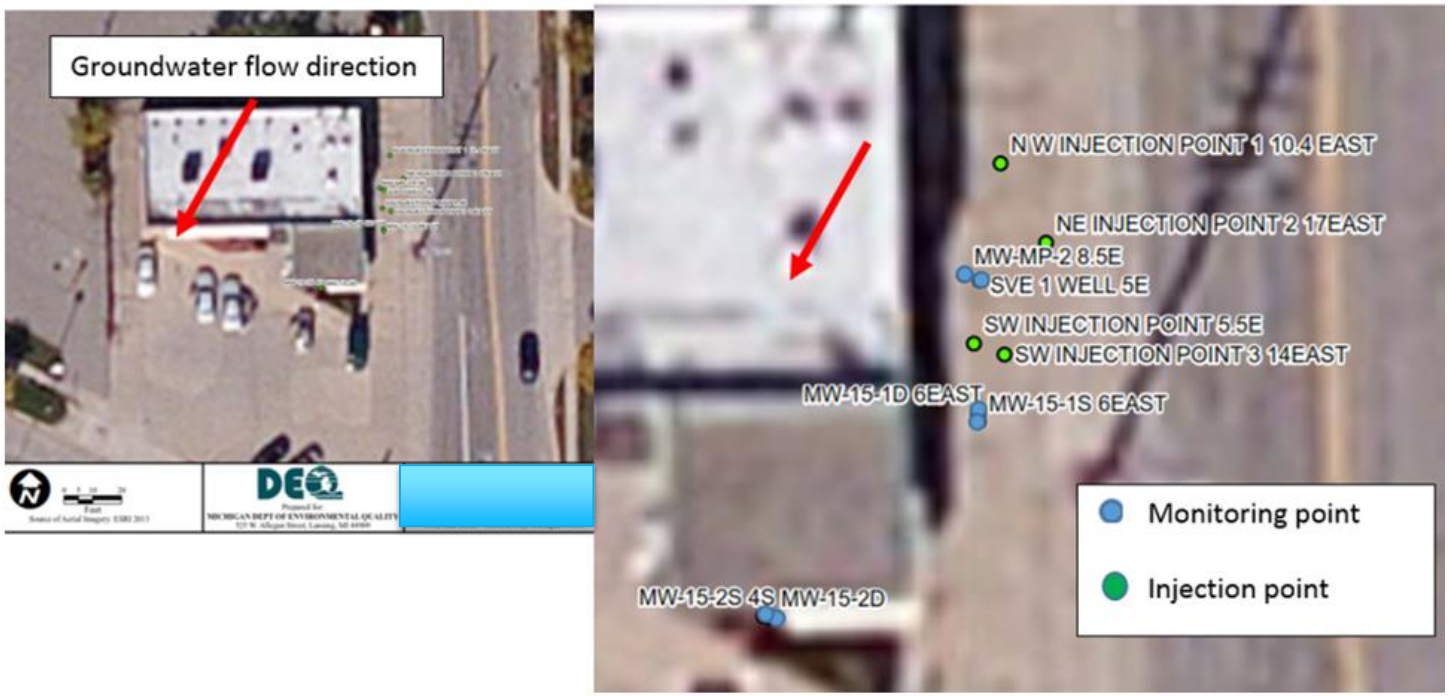
- Active Dry Cleaning Facility, southern Michigan
- Shallow groundwater 5 ft bgs confined by a clay layer at 12 ft bgs.
- PCE (max. 35 ppm) and TCE (max. 14 ppm) along with an accumulation of anaerobic catabolites *cis* 1,2-DCE (max. 25 ppm) and some VC (max. 4 ppm).
- Source area up to 70 ppm total CVOCs
- Groundwater migrates through a sandy aquifer into a damaged storm sewer.
- A sanitary sewer feeder from the active dry cleaner exacerbating the PCE migration problem by allowing warm water with potential contaminants and surfactants to enter the groundwater.
- Consultant and Agency selected Provect-IR over conventional ERD and ISCR reagents known to induce methane production.

Provect-IR Field Pilot: Focused on CH4

Table 1. Summary of Provect-IR Applications for Field-Scale Pilot Test

Depth Interval (ft bgs)	Amount of Provect-IR Injected (lb)			
	Point 1	Point 2	Point 3	Point 4
11 to 12	75	100	100	150
9 to 10	75	100	100	100
7 to 8	50	50	50	50
TOTAL	200	250	250	300

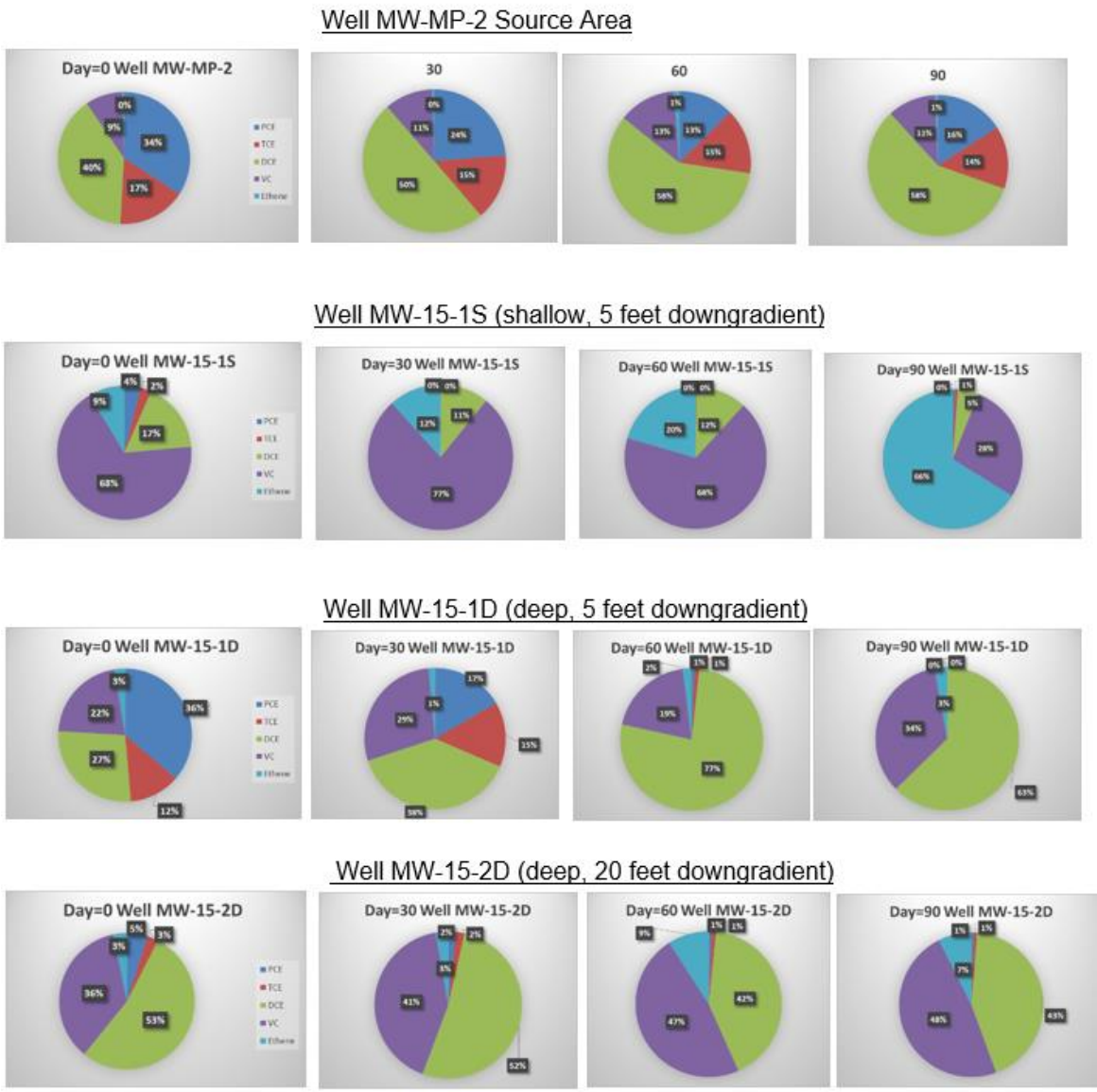
Figure 1. Pilot Test Area



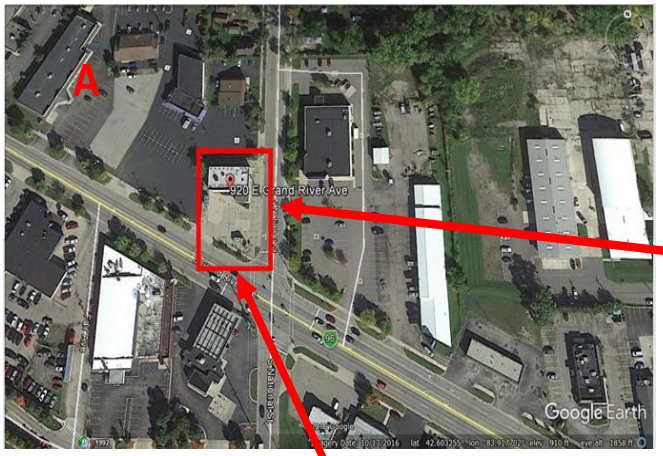
Active Dry Cleaner – 90 Days

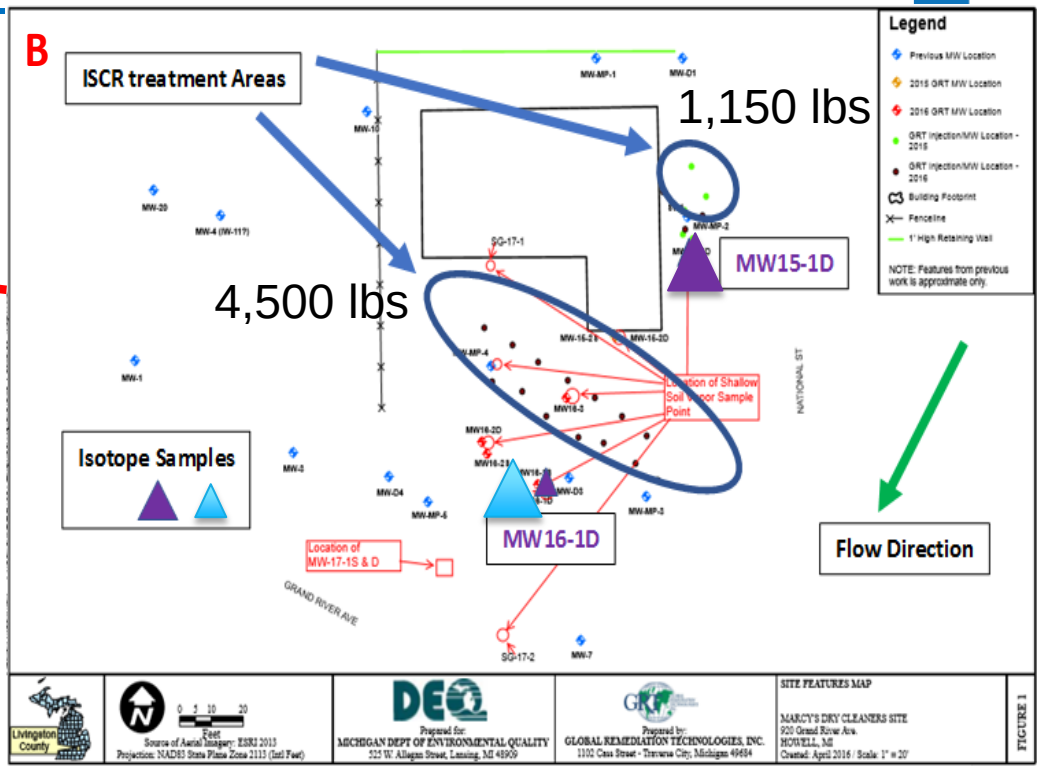
- WEIGHT OF EVIDENCE

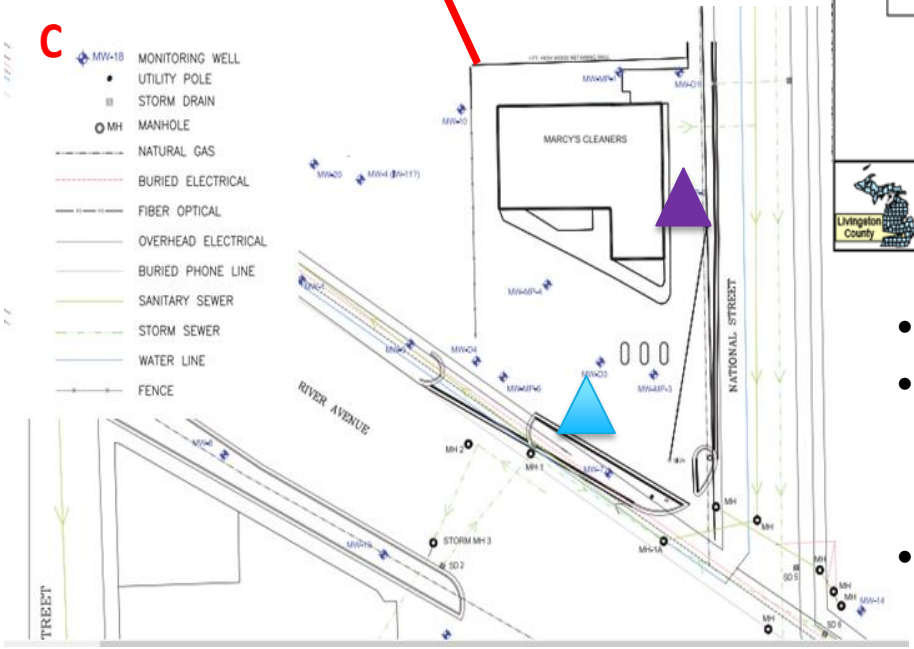
- Total CVOCs reduced by 62 to >99%
- No accumulation of DCE or VC as dead-end catabolites
- No groundwater methane accumulation during any sampling event (ranged from 1.7 mg/L @ Time=0 to a high of 2.2 mg/L @ 60 days after Provect-IR additions.
- Soil gas methane baseline <20 ppmv to a high of 94 ppmv 30 days after the injection event (Day 60 and Day 90 <20 ppm)



Phase II Implementation (March 2016)







- *>90% PCE removal, no DCE/VC stall*
- *ca*. 9 months post Provect-IR treatment CH4 from 5 to <10 mg/L was observed at two well locations ▲▲
- >200,000 ppmV in soil gas at MW-16S ▲

Origin of CH4 After 9 Months

$\Delta^{14}C$ and $\delta^{13}C$ Data Review

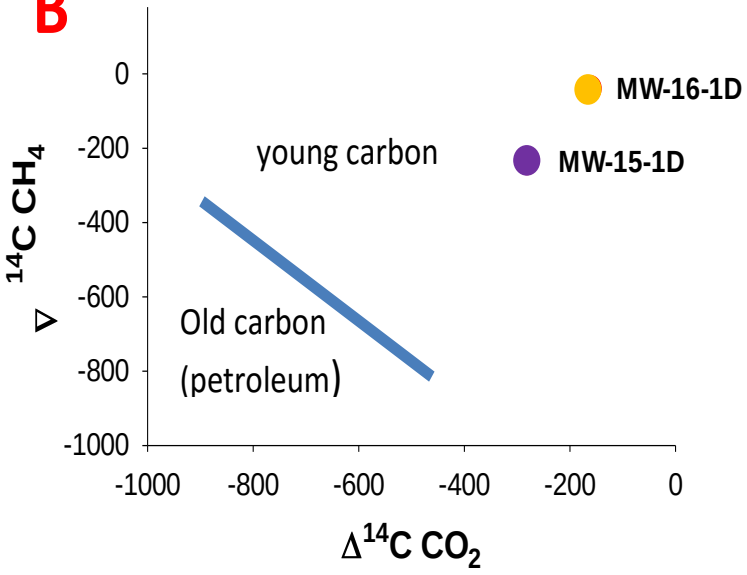
A

Stable Carbon and Radiocarbon Data Summary

Sample Identification	Type	F Modern	Fm Err	Age (years)	Age Err	$\delta^{13}C$	$\Delta^{14}C$
MW-16-1D-CO2, groundwater	CO2	0.8469	0.0020	1,340	20	-71.48	-159.96
MW-15-1D-CO2, groundwater	CO2	0.7261	0.0024	2,570	25	-16.65	-279.81
MW-16-1D-CH4, groundwater	CH4	0.9669	0.0019	270	15	-57.96	-40.95
MW-15-1D-CH4, groundwater	CH4	0.7677	0.0016	2,120	15	-60.61	-238.54

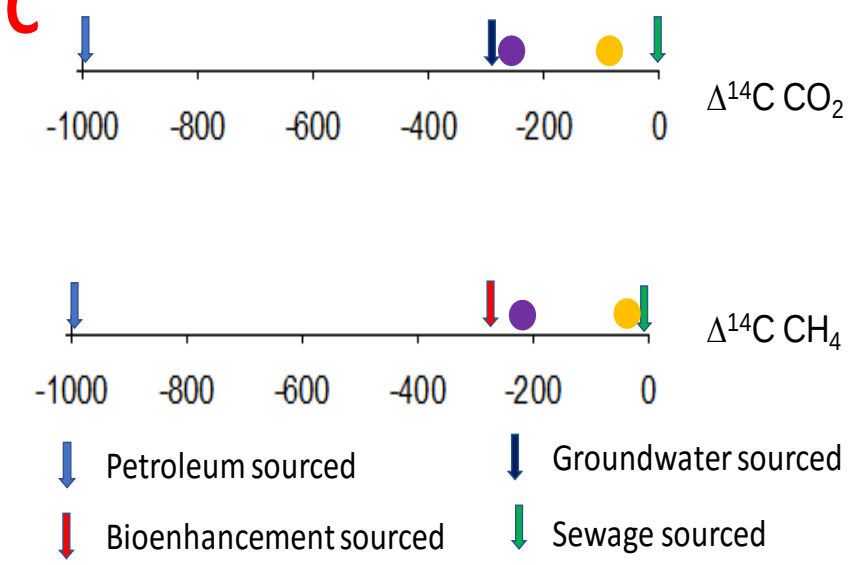
B

Radiocarbon Isotope CH_4 and CO_2 Data



C

Potential CO_2 and CH_4 $\Delta^{14}C$ Source Values



So, is Methanogenesis Important?

Some say…	Response
✓ We never see problems	mostly because we don't look
✓ My site is remote	ignores efficiency issue
✓ Our amendments don't make methane	then they are not working well
✓ It's bad to stop methanogens	it's beneficial to control them
✓ Our clients don't worry about it	then YOU better worry about it…
✓ We will just add more reagent	$$ not acceptable to our clients

You decide

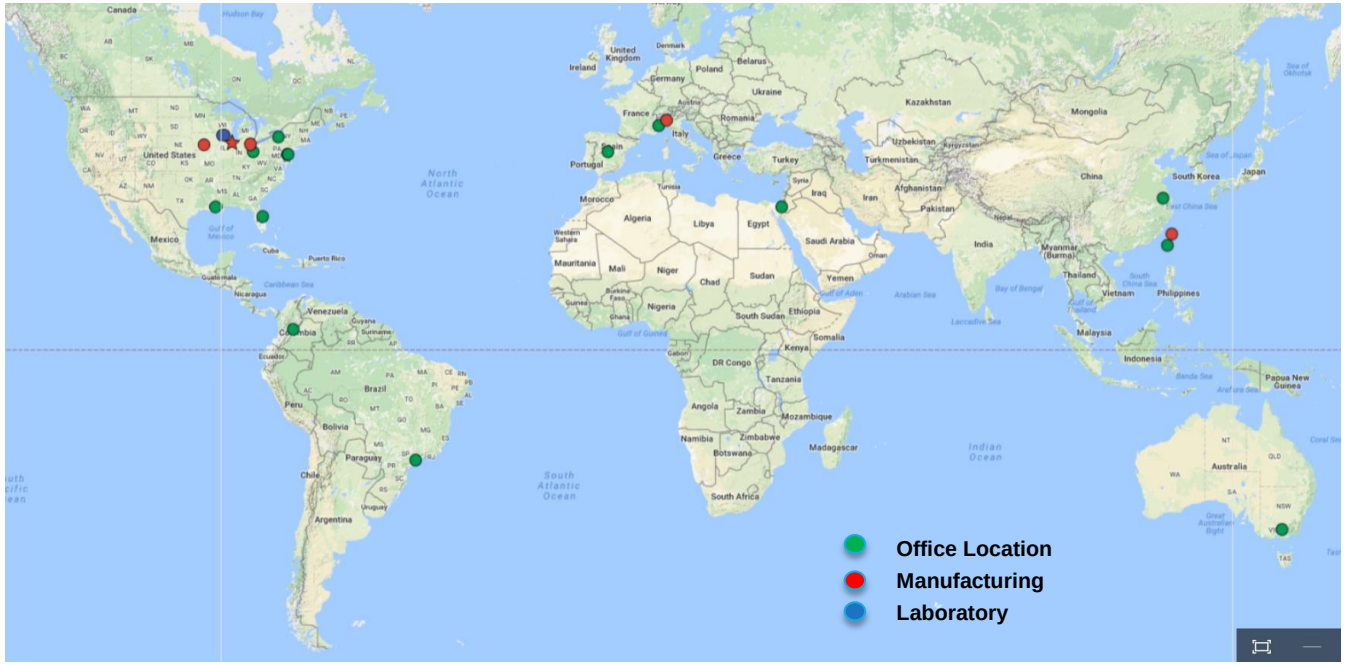
- ✓ Look at your own data
- ✓ Evaluate your site conditions
- ✓ Co-metabolism (IRB, SRB) of CHCs is slow, and mostly stalls at DCE/VC
- ✓ Experienced clients understand the value added
- ✓ What do your regulators say …

Summary

- Natural statins in RYR, essential plant oils and other materials can be used to effectively and specifically control methanogenic activity
- The methane control technology has been integrated into various products designed for the environmental remediation industry
  - **Provect-CH4®** ERD Supplement / Methane Inhibitor
  - **ERD-CH4® Ole Ego™** Liquid, Antimethanogenic ERD Reagent
  - **Provect-IR®** Solid, Antimethanogenic ISCR Reagent
  - **Provect-IRM®** Antimethanogenic ISCR Reagent for Metals
  - **AquaGate®-CH4™** Antimethanogenic *In Situ* Sediment Capping Technology
  - **EZVI-CH4™** Antimethanogenic Source Area / DNAPL Treatment
- The main benefit is improved performance = “better gas mileage”
- Other potential benefits relate to safety, regulatory compliance, and sustainability

Provectus Environmental Products

- Complimentary Site Evaluation
- Complimentary review of quarterly field performance data for 1 year with every project
- Laboratory Treatability Studies
- Turn-Key, Pay-for-Performance Contracting Options
- Project Specific Guarantees and Warranties



- USA (Illinois, New York, Ohio, Pennsylvania, Louisiana)
- Brazil, China, Colombia, Israel, Italy, Spain and Taiwan